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Mechanisms of Pathogen-Derived Resistance to Viruses in Transgenic Plants

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INTRODUCTION

In 1985, Sanford and Johnston developed the simple and elegant concept of parasite- or pathogen-derived resistance (Sanford and Johnston, 1985). Subsequently, there have been numerous attempts to generate virus resistance in transgenic plants based on this concept through the expression of virus-derived genes or genome fragments (Beachy, 1993; Wilson, 1993; Baulcombe, 1994b; Lomonossoff, 1995). Many of these attempts have been successful, and some have led to the development of virus-resistant potato and squash cultivars for commercial application.

In this article, the major emphasis is on the mechanisms of pathogen-derived resistance rather than on the practicalities of using this technology for crop improvement. These mechanisms have proven difficult to unravel largely because a resistance mechanism related to transgene silencing can override the direct phenotype of virus-derived transgenes. The examples discussed first are those in which it is clear whether the mechanism involves gene silencing. In later sections, some less well understood examples are reviewed. The final section anticipates likely future developments in pathogen-derived resistance.

VIRUS RESISTANCE THAT DOES NOT DEPEND ON GENE SILENCING

Coat Protein-Mediated Protection

The coat protein (CP) gene of tobacco mosaic virus (TMV) was used in the first demonstration of virus-derived resistance in transgenic plants (Powell-Abel et al., 1986). In these experiments, transgenic tobacco plants expressing high levels of the TMV CP were more resistant to TMV virions than to TMV RNA inocula. Based on this observation, it was suggested that CP-mediated protection (CPMP) against TMV was through the inhibition of virion disassembly in the initially infected cells (Register and Beachy, 1988). It was proposed that RNA inocula could overcome the resistance because disassembly was not required to establish infection by naked RNA. Once established, the viral RNA could spread unimpaired in inoculated leaves of transgenic plants because TMV RNA does not need to be encapsidated (and therefore disassembled) as it moves between cells.

Several lines of evidence are consistent with resistance to initial infection in CPMP. For example, targeted expression of CP in restricted cell types revealed that CPMP depends on CP production in the initially infected epidermal cells (Reimann-Philipp and Beachy, 1993). Moreover, there was no resistance when the transgenic promoters directed CP expression in leaf mesophyll cells, which become infected after epidermal cells. A second direct test of the disassembly hypothesis involved encapsulation of β-glucuronidase (GUS) mRNA to produce TMV-like particles (pseudovirions). These GUS pseudovirions were inoculated to protoplasts of plants exhibiting CPMP and to those of nontransgenic plants (Osbourn et al., 1989). The production of GUS was much lower in the CPMP protoplasts than in protoplasts of nontransgenic plants, which is consistent with an inhibition of (pseudo)virion disassembly (Osbourn et al., 1989). A third test of the disassembly hypothesis was the demonstration that transgenic expression of mutant and recombinant forms of the TMV CP conferred resistance to TMV (Clark et al., 1995a, 1995b). This resistance was associated with the potential of mutant CP to interact with the CP subunits of inoculated wild-type virions. It was suggested that this interaction could inhibit virion disassembly.

These data were all consistent with inhibition of virion disassembly in CP transgenic plants but did not rule out the possibility that later stages in the virus infection cycle, such as systemic virus movement, may also be suppressed. Thus, a grafting experiment was performed to test the influence of CPMP on movement of TMV through the vascular system. A segment of a transgenic plant exhibiting CPMP was grafted between a stock and a scion of a TMV-susceptible, nontransgenic plant. The movement of TMV through the grafted segment was inhibited, which is consistent with an effect of CPMP on vascular movement (Wisniewski et al., 1990). However, it is likely that movement of TMV in the vascular system involves virion assembly and disassembly (Saito et al., 1990). Thus, the suppression of vascular movement could be a secondary consequence of the inhibition of virion disassembly.

Additional evidence supporting the hypothesis that CPMP operates at the level of virion disassembly is based on experiments with alfalfa mosaic virus (AlMV). However, in this

instance, the resistance mechanisms may be more complex than CPMP against TMV. In lines producing a low level of AlMV CP, the resistance was effective only against AlMV virion inocula. Conversely, in transgenic lines producing either a mutant CP or high levels of wild-type CP (Taschner et al., 1994), the CPMP against AlMV was effective against both RNA and virion inocula. As with TMV CPMP, the influence of the type of inoculum suggests that CPMP against AlMV acts on at least two levels. One level would be inhibition of virion disassembly and would require low-level expression of the CP. The second level of resistance, which is exhibited in lines with mutant or high levels of CP, could involve any interaction of the CP required for the virus infection cycle. These could include interactions with the viral RNA necessary for assembly or virus replication (Reusken et al., 1994), or with host plant receptors of the CP (Taschner et al., 1994).

The CPMP against potato virus X (PVX) is also effective against both RNA and virion inocula. As with AlMV resistance, there is the potential for disruption of any interaction of the CP that is required for the replication and spread of PVX in the infected plant (Hemenway et al., 1988). It was suggested that an interaction of transgenic CP with the PVX origin of assembly could lead to resistance. In potexviruses, the origin of assembly is likely to be in the 5′ region of the viral genome (Sit et al., 1994) so that a CP interaction would have the potential to suppress translation of the viral RNA-dependent RNA polymerase (RdRp) that is encoded in the 5′-most open reading frame (ORF). However, it is also possible that CPMP inhibits cell-to-cell movement of PVX, for which CP is an essential cofactor (Chapman et al., 1992).

Movement Protein-Mediated Protection

Most examples of CPMP are based on the transgenic expression of wild-type CP genes. However, it is also possible to engineer pathogen-derived resistance with dominant negative mutant forms of viral genes. The effectiveness of this strategy was illustrated by the transgenic expression of viral movement proteins (MP), which conferred resistance only when the transgene specified a dysfunctional MP (Lapidot et al., 1993; Malyshenko et al., 1993). Transgenic expression of a functional MP either had no effect on virus infection or increased susceptibility (Ziegler-Graff et al., 1991).

Resistance conferred by transgenic expression of a dysfunctional TMV MP is likely due to competition for plasmodesmatal binding sites between the mutant MP and the wild-type MP of the inoculated virus (Lapidot et al., 1993). An interesting and potentially useful attribute of MP-mediated protection (MPMP) is the broad spectrum efficacy of the resistance mechanism. The protection conferred by the mutant MP of TMV, for example, mediates resistance to viruses of the potex-, cucumo-, and tobraviral groups in addition to the tobamoviruses (Cooper et al., 1995). Similarly, transgenic expression of the brome mosaic virus (BMV) MP in a nonhost plant conferred resistance to TMV (Malyshenko et al., 1993). These examples of broad spectrum resistance indicate that MPs of several different viruses may interact with the same plasmodesmatal components (see Carrington et al., 1996, in this issue, for a review of virus movement).

In the potex-, carla-, hordei-, and furovirus groups, three MPs are encoded by a series of overlapping reading frames referred to as the triple gene block (TGB; Petty and Jackson, 1990; Beck et al., 1991). Transgenic expression of a mutant TGB protein confers resistance against a narrower range of viruses than does the TMV MPMP (Beck et al., 1994). This narrower specificity may indicate that the TGB proteins do not interact with the plasmodesmata in precisely the same way as does the TMV MP.

RNA (or DNA)-Mediated Resistance

In the previously described examples, resistance was conferred by proteins encoded by the transgene. In other examples, pathogen-derived resistance was due to direct inhibition of the viral infection cycle by the transgene itself or by its RNA transcript. This RNA (or DNA)-mediated resistance could operate, for example, if the transgenic nucleic acid acts as a decoy molecule. This decoy would compete with the viral genome to redirect host- or viral-encoded proteins into interactions that would be nonproductive for replication or spread of the virus in the infected plant. This type of competitive inhibition could operate when the transgene specified a defective-interfering (DI) RNA (or DNA), encompassed a *cis*-acting element in the viral genome, or was derived from a satellite RNA.

Examples of pathogen-derived resistance that are probably the result of decoy action of the transgenic RNA include satellite RNA–mediated resistance (Gerlach et al., 1987; Harrison et al., 1987), resistance to geminiviruses conferred by a transgenic DI DNA (Stanley et al., 1990), and resistance to cymbidium ringspot virus due to transgenic expression of a replicable DI RNA (Kollàr et al., 1993). It is likely that resistance from a transgenically expressed 3′ DI RNA of turnip yellow mosaic virus is conferred through direct competitive inhibition with the viral genome (Zaccomer et al., 1993).

RESISTANCE MECHANISMS DEPENDENT ON GENE SILENCING

Gene Silencing and Pathogen-Derived Resistance

The examples of pathogen-derived resistance discussed above are those in which it is clear that the phenotype of the transgenic plants is not complicated by gene silencing. By contrast, the following examples illustrate that gene silencing is involved in and is responsible for the resistance mechanism.

The realization that gene silencing is involved in pathogen-derived resistance has evolved through several stages. Initially, there was the counterintuitive finding that the level of RNA ac-

cumulating from a virus-derived transgene did not always correlate with the degree of virus resistance (Lawson et al., 1990). Equally confusing was the subsequent finding that resistance was conferred by modified viral transgenes that encoded untranslatable RNAs (De Haan et al., 1992; Lindbo and Dougherty, 1992b; Van der Vlugt et al., 1992). These findings were reconciled in an analysis of transgenic resistance against tobacco etch virus (TEV). In tobacco plants exhibiting an extreme level of resistance to TEV, there was an association between resistance and post-transcriptional suppression of transgene expression (Lindbo et al., 1993), and it was proposed that these two phenomena were caused by the same mechanism. This proposal was substantiated by the findings that gene silencing with nonviral transgenes is due to a post-transcriptional mechanism (Ingelbrecht et al., 1994; de Carvalho Niebel et al., 1995). This post-transcriptional mechanism operates at the RNA level and would therefore have the potential to suppress the accumulation of viral RNA that shares sequence identity with the silenced transgene.

A link between pathogen-derived resistance and transgene silencing was confirmed by genetical tests with plants exhibiting resistance to PVX and potato virus Y (PVY; Mueller et al., 1995; Goodwin et al., 1996). These tests showed that viral transgenes conferring a virus resistance phenotype also silenced the expression of homologous transgenes through a post-transcriptional mechanism. Additionally, in experiments with a PVX vector construct carrying GUS sequences (PVX-*GUS*), it was confirmed that post-transcriptional gene silencing of a nonviral transgene (GUS) had the potential to suppress the accumulation of viral RNA. For virus suppression, it was necessary only that the viral genome have some sequence identity to the transgene (English et al., 1996). This virus resistance mechanism is referred to as homology-dependent resistance to reflect the relationship with homology-dependent gene silencing (Mueller et al., 1995).

Antisense RNA in Homology-Dependent Resistance

The experiments described above led to the development of conceptual models of post-transcriptional gene silencing and pathogen-derived resistance to viruses. These models proposed a mechanism for suppressing the accumulation of nucleus-derived RNA (gene silencing) and virus-derived RNAs with homology to the transgene (resistance; Lindbo et al., 1993; Mueller et al., 1995; English et al., 1996; Goodwin et al., 1996). Such a mechanism would require a high degree of sequence specificity, because resistance is highly strain specific. For example, homology-dependent resistance to PVX was ineffective against strains that differed from the transgene by as little as 22% at the nucleotide level (Mueller et al., 1995). To account for this sequence specificity, it has been suggested that there are base-pairing interactions involving an RNA produced from the transgene. This transgenic RNA would interact with viral RNA to account for homology-dependent virus resistance and with nucleus-derived RNA to account for homology-dependent gene silencing (Mueller et al., 1995). The likely nature of this interaction and how it could result in the suppression of both nucleus- and virus-derived RNA are considered in the remainder of this section.

In principle, the interaction leading to suppression of viral RNA could involve base pairing of the sense RNA transcript of the transgene and the negative strand of the viral RNA, which is produced as an intermediate in the replication cycle of most viral RNAs. However, in gene silencing there would have to be an interaction between the RNA from the silencing transgene and the homologous RNA of the silenced genes. Because both RNAs would have the same polarity, it is not obvious how they could interact by base pairing. A more plausible explanation is that the interaction is indirect and, as proposed originally by Lindbo et al. (1993), is mediated by an RNA complement of the transgene RNA. This antisense RNA could be produced by an RdRp encoded in the host genome by using the transgene RNA as a template (Schiebel et al., 1993a, 1993b) and would have the potential to base pair with both the transgenic and viral RNAs (Figure 1A).

The only direct experimental support for antisense RNA in post-transcriptional gene silencing and virus resistance is the finding that RNA is copied into the complementary strand when electroporated into barley protoplasts (Huntley and Hall, 1993). In addition, a host RdRp has been purified and characterized biochemically (Schiebel et al., 1993a, 1993b). Until now, the predicted antisense RNA has not been detected in plants exhibiting homology-dependent resistance. However, it may be that the methods used to search for this hypothetical antisense RNA would not have detected the small or heterodisperse RNAs that are the likely products of the host-encoded RdRp (Schiebel et al., 1993a, 1993b).

There are several ways in which the formation of duplex RNA could influence accumulation of host and viral RNAs to cause virus resistance and gene silencing (Figure 1B). For example, the base-paired region may render the duplex RNA susceptible to degradation by RNases specific for double-stranded RNA (Nicholson, 1996). The base-paired region could also arrest translation and consequently have an indirect effect on RNA stability (Green, 1993). These effects on RNase susceptibility and translation could cause reduced accumulation of both nucleus- and virus-derived RNAs (Figure 1B). In addition, there could be effects that would only be specific for virus-derived RNAs. For example, hybrid arrest of translation could have a direct effect on viral RNA accumulation if the translation product were a cofactor of viral RNA accumulation. Furthermore, if the base-paired region is required in *cis* for replication of viral RNA, the formation of a duplex with an antisense RNA could inhibit virus replication. This potential for diverse effects of RNA duplex formation, which is summarized in Figure 1B, extends a previous model that invoked only RNase susceptibility as the consequence of base pairing between an antisense RNA and homologous virus- or nucleus-derived RNAs (Lindbo et al., 1993; Goodwin et al., 1996).

The proposed involvement of antisense RNA can be considered as part of the response phase of homology-dependent

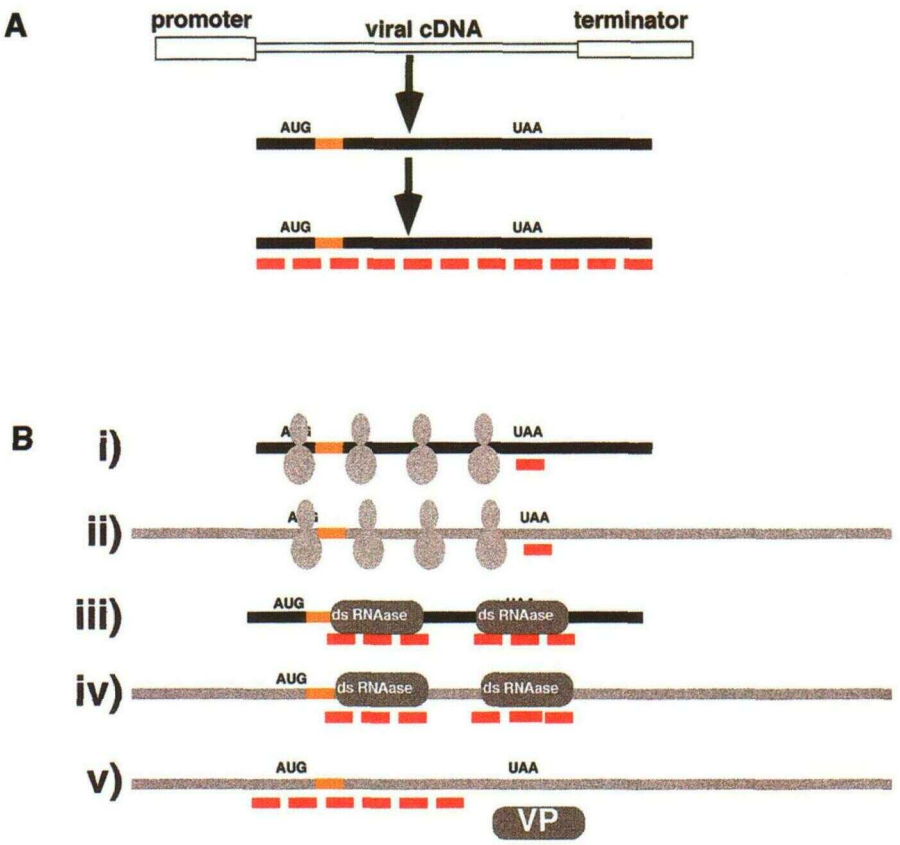

Figure 1. Antisense RNA and Homology-Dependent Resistance.

(A) Production of antisense viral RNA fragments. The model of Lindbo et al. (1993) in which it is proposed that a viral cDNA transgene is transcribed and then copied by a host-encoded RdRp into small fragments of antisense RNA (red) is shown. The viral cDNA may encompass *cis*-acting elements of the viral genome (yellow) that are required for either replication, encapsidation, or cell-to-cell movement.

(B) Potential molecular mechanisms leading to antisense RNA-mediated homology-dependent resistance. Antisense RNA fragments (red) bind to homologous viral RNA (gray) or transgene RNA (black) with varying consequences, depending on whether the target RNA is virus- or transgene-derived, on the function of the protein encoded in the hybridizing region, or on whether the RNA has roles other than as a protein-encoding template. *(i) and (ii) Antisense RNA binds to transgene (i) and/or viral (ii) RNA, blocking translation and thereby destabilizing the transcript. If* the encoded protein is required for virus replication, the accumulation of viral genomic RNA is inhibited; (iii) and (iv), binding of antisense RNA fragments to transgene (iii) and viral (iv) RNAs recruits a double-strand (ds) specific RNase, which degrades the target transcript; and (v), antisense RNA binding masks a *cis*-acting element in the viral genome, preventing its interaction with a virus-encoded protein (VP). The VP–RNA interaction may be necessary for virus replication, movement, or other essential phases of the infection cycle. The inhibition of this interaction could therefore prevent virus accumulation in the inoculated plant.

resistance. A separate issue concerns the activation phase: why do some lines exhibit homology-dependent resistance and gene silencing, whereas others carrying the same construct do not? The current models that explain this observation fall into the following two categories: (1) those involving a quantitative factor in which there is a threshold level of transgene expression that activates homology-dependent resistance (Figures 2A and 2B; Lindbo et al., 1993; Smith et al., 1994; Goodwin et al., 1996); and (2) those involving a qualitative factor that affects production of an aberrant RNA in lines exhibiting homology-dependent resistance (Figures 2A and 2C; Mueller et al., 1995; English et al., 1996). These models are evaluated in the following two sections.

Recovery, Transgene Dosage, and the Threshold Model

In the most extreme examples, homology-dependent resistance is a constitutive trait: the transgenic plants are resistant at all stages of development and before virus inoculation. However, there are also intermediate examples in which full resistance was only induced a week or more postinoculation with a virus that was similar to the transgene at the sequence level. This

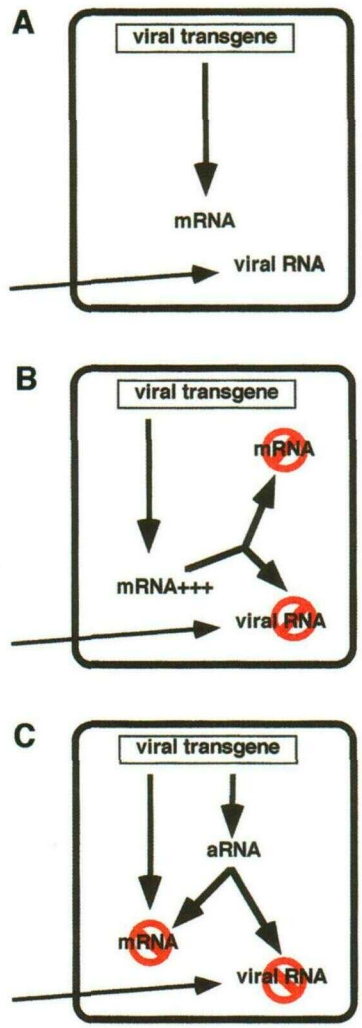

Figure 2. Initiation of Homology-Dependent Resistance.

(A) Susceptible transgenic plant. In most transgenic lines, a viral transgene is transcribed, the corresponding mRNA (and protein; not shown) accumulates, and the plant is susceptible to the transgene-homologous virus.

(B) The threshold model for homology-dependent resistance. In this model, in the minority of transgenic lines that exhibit pathogen-derived resistance, there is a mechanism that detects high levels of the transgene mRNA (+++). This mechanism suppresses (red symbol) accumulation of nucleus- and virus-derived RNAs that are homologous to the transgene.

(C) The qualitative model for homology-dependent resistance. This model does not posit a mechanism that monitors the level of transgene expression. Instead, it requires the production of an aberrant RNA (aRNA) that activates the suppression of viral and transgene RNA accumulation.

induced form of resistance has been referred to as "recovery," because the virus-infected plants undergo a transition from a diseased to a healthy appearance (Lindbo et al., 1993; Swaney et al., 1995; Goodwin et al., 1996). In these experiments, the inducing virus was absent from the recovered parts of the plant. Moreover, post-transcriptional silencing of the viral transgene occurred coincident with the development of resistance (Lindbo et al., 1993). It was proposed that homology-dependent resistance and gene silencing were only induced after the combined levels of viral and transgene RNA surpassed a threshold level (Figure 2B; Lindbo et al., 1993).

Other evidence supporting threshold-activated gene silencing is based on analyses of transgene transcription and transgene dosage in lines transformed with the CP ORF of PVY or TEV. In these cases, resistance was generally more extreme in lines with higher levels of transcription or with higher transgene dosage (Lindbo et al., 1993; Goodwin et al., 1996). It was proposed that the increased transgene dosage would take transcript levels over the threshold and that homology-dependent resistance would be activated as a secondary consequence.

The relationship between transgene dosage and homology-dependent resistance or gene silencing is also found with resistance to PVX or tomato spotted wilt virus (Mueller et al., 1995; Pang et al., 1996). In one example of homology-dependent resistance to PVX, the resistance phenotype required at least three transgene loci. Lines carrying one or two transgene loci were susceptible and accumulated high levels of the transgene RNA (Mueller et al., 1995).

Aberrant RNA

Although the transgene dosage data and the recovery phenomenon can be interpreted in terms of a threshold model, data from several systems are inconsistent with an RNA threshold of post-transcriptional gene silencing and homology-dependent resistance. For example, in lines displaying RNA homology–dependent resistance to isolates of PVX, the transgene transcription level was as high as, but not higher than, that in susceptible lines transformed with identical constructs (Mueller et al., 1995; English et al., 1996). A more extreme example is that of petunia lines exhibiting post-transcriptional silencing of chalcone synthase through a mechanism that is likely to be similar to homology-dependent resistance. The chalcone synthase transgene constructs responsible for this effect did not have a promoter and were not transcribed at detectable levels as would be required by the threshold model (Van Blokland et al., 1994). Furthermore, the threshold hypothesis does not explain the delayed onset of resistance in plants displaying the recovery phenotype. If a simple quantitative RNA threshold were the major limiting factor, it would be expected that resistance and transgene silencing associated with recovery would develop throughout the infected parts of the plant. In fact, there was no resistance in the inoculated and lower systemic leaves of the recovered plants. Homology-dependent

resistance and gene silencing in these plants developed only in leaves in the upper parts of the plants, well above the inoculated leaf (Lindbo et al., 1993).

To accommodate these problems, an alternative model has been proposed in which the crucial factor affecting homology-dependent resistance and post-transcriptional gene silencing would be qualitative rather than quantitative (English et al., 1996). According to this qualitative model, a transgene would have gene silencing potential or confer homology-dependent resistance only if it is transcribed to produce an aberrant RNA (Figure 2C). The precise nature of the aberration in this RNA was not defined. However, it may be a feature that would render the RNA a preferred template for the production of antisense RNA by the host-encoded RdRp, as discussed above (English et al., 1996).

An analysis of gene silencing in the fungus *Ascobolus immersus* indicated how aberrant RNA may be produced. In this fungus, the presence of transgenic copies or partial copies of the methionine biosynthetic (*met2*) locus caused the endogenous *met2* gene to become methylated over regions that were duplicated in the transgenes. Transcription of *met2* RNA was terminated in the methylated regions of the endogenous gene to produce aberrant, truncated RNAs. It was suggested that methylation was the consequence of an ectopic interaction between the transgene and a homologous region of the corresponding endogenous gene (Barry et al., 1993).

Several observations are consistent with the hypothesis that homology-dependent resistance and post-transcriptional gene silencing may be initiated in a manner related to the silencing of *met2* in *A. immersus* (Baulcombe and English, 1996). These include the finding that the resistance and silencing phenotypes are often associated with multiple copies of transgenes. The presence of multiple transgenes would create an increased likelihood of ectopic pairing (Mueller et al., 1995; Goodwin et al., 1996; Pang et al., 1996). There are also many reports of an association between post-transcriptional gene silencing, homology-dependent resistance, and transgene methylation (Hobbs et al., 1990, 1993; Ingelbrecht et al., 1994; Smith et al., 1994; English et al., 1996; T. Sijen and J. Wellink, personal communication).

Recovery and RNA-DNA Interactions

To accommodate the recovery phenomenon in the aberrant RNA model of gene silencing, it has been proposed that there could be an interaction between viral RNA and homologous transgene DNA (Baulcombe and English, 1996). Such an interaction would lead to methylation or other reversible modifications of the transgene, which in turn would lead to the production of an aberrant RNA that could mediate the resistance mechanism and gene silencing. A precedent for RNA-directed methylation of DNA has been provided by studies of transgenic plants carrying sequences homologous to the potato spindle viroid. In these experiments, the viroid transgene sequences were specifically methylated whenever there was an accumulation of the potato spindle viroid in the nuclei of the transgenic plants (Wassenegger et al., 1994).

How would viral RNA interact with homologous host transgene DNA? In most parts of virus-infected plants, there is no opportunity for such an interaction to occur, because most RNA viruses accumulate in the cytoplasm. However, in developing leaves, the transient breakdown of the nuclear membrane that occurs during cell division could allow an interaction between cytoplasmic viral RNA and homologous nuclear DNA. The requirement that this RNA–DNA interaction takes place in leaf primordia would explain why the recovered state developed only in the upper leaves of the transgenic plants (Lindbo et al., 1993). The older lower leaves would have been infected after the phase of cell division and too late for the interaction of viral RNA with homologous nuclear DNA.

A phenomenon similar to the recovery of transgenic plants developed when TMV vector constructs were inoculated into *Nicotiana benthamiana* (Kumagai et al., 1995). The TMV constructs were modified to carry small regions of the host plant phytoene desaturase sequence. Phytoene desaturase is a carotenogenic enzyme and is essential for protection against photobleaching. In the inoculated and lower systemic leaves, the viral symptoms were similar to those caused by the unmodified TMV, but in the upper parts of the plant, the leaves were bleached (Kumagai et al., 1995). These symptoms could be explained by viral RNA interactions with homologous DNA in dividing cells, as suggested above for plants exhibiting recovery to potyviruses. The RNA–DNA interaction could lead to silencing of the host gene and the consequent bleaching phenotype.

An RNA–DNA interaction may also explain the activation of post-transcriptional gene silencing or homology-dependent resistance in lines with single-copy transgene loci. In these lines, the absence of repeated transgenes meant that there was no opportunity for ectopic pairing of homologous DNA leading to DNA methylation and aberrant RNA formation (Elmayan and Vaucheret, 1996; Goodwin et al., 1996). Perhaps, in these examples, the transgenic RNA accumulated in the nucleus, interacted with the homologous DNA, and initiated the production of aberrant RNA, as proposed for the recovery phenomenon.

The proposals that DNA–DNA and DNA–RNA interactions of the transgene may facilitate homology-dependent resistance through DNA methylation are easily testable. For example, it should be possible to extend the correlation of DNA methylation and resistance by direct analysis of methylated residues in the viral cDNA sequences. Moreover, the methylation status of transgene DNA could be manipulated by use of genetically modified plant lines with low levels of DNA methylation enzymes (Kakutani et al., 1995; Finnegan et al., 1996). In addition, it may be useful to evaluate the chromatin structure of the transgene. It is possible that chromatin changes, which can also be caused by ectopic DNA interactions (Dorer and Henikoff, 1994), could influence transcription and, like DNA methylation, be transmitted somatically in a reversible manner.

ADDITIONAL EXAMPLES OF PATHOGEN-DERIVED RESISTANCE

The examples of pathogen-derived resistance discussed in the previous sections provide a conceptual outline for resistance mechanisms. In the examples discussed below, the situation is less straightforward. These examples may involve variations of homology-dependent resistance that may in due course help to reveal the nature of the underlying mechanism. Alternatively, these examples may reflect unexpected complications in the interactions between viruses and their plant hosts. It is also possible, as described for transgenic resistance to members of the tospovirus group, that homology-dependent resistance and other mechanisms could be operating simultaneously (Pang et al., 1993).

Antisense RNA-Mediated Protection

Reports of antisense RNA–mediated protection fall into two categories. In some instances, when resistance is weak, protection is probably related to direct RNA-mediated resistance, in which the transgenic RNA either serves as a decoy molecule or hybridizes with, and thereby neutralizes, *cis*-acting elements in the viral genome. Examples in this category include resistance resulting from transgenic expression of the antisense RNA of the 5′ end of the TMV genome (Nelson et al., 1993) and the low level of resistance observed when genes of TMV, PVX, cucumber mosaic virus (CMV), and bean yellow mosaic virus are transgenically expressed as antisense RNA (Cuozzo et al., 1988; Hemenway et al., 1988; Powell et al., 1989; de Feyter et al., 1996).

In the second category, a high level of resistance was seen (Kawchuk et al., 1991; Lindbo and Dougherty, 1992a; Ravelonandro et al., 1993; Yepes et al., 1996). This type of resistance had many of the attributes of homology-dependent resistance, including strain specificity and, with potyviral transgenes, a recovery phenotype (Ravelonandro et al., 1993). Therefore, it is likely that homology-dependent resistance is activated by antisense transgenes, although less efficiently than it is with sense transgenes.

Replicase-Mediated Resistance

In plants carrying a transgene derived from the replicase genes of PVX (Mueller et al., 1995), cowpea mosaic virus (Sijen et al., 1995), and pepper mild mottle tobamovirus (Tenllado et al., 1995, 1996), it is clear that there was RNA-mediated, homology-dependent resistance. In other examples, it is likely that the replicase transgene conferred homology-dependent resistance, although the data are not conclusive (Macfarlane and Davies, 1992; Rubino et al., 1993; Rubino and Russo, 1995). In the one example of replicase-mediated resistance in which gene silencing could be ruled out completely, the TMV replicase transgene was modified by the unintended insertion of a bacterial transposable element during propagation in bacterial hosts (Donson et al., 1993). It is likely that the site of transposon insertion and the properties of the replicase fusion protein product of the transgene were crucial factors in this resistance mechanism. Plants expressing this construct were also resistant to tobamoviruses only slightly similar to TMV, suggesting that gene silencing mechanisms were not involved (Donson et al., 1993).

In other examples of transgenic resistance mediated by viral replicase transgenes, it is not clear whether homology-dependent resistance was involved (Baulcombe, 1994a). In the case of plants transformed with the ORF specifying the 54-kD replicase protein of TMV, there was strong resistance to TMV (Golemboski et al., 1990). This resistance, like the homology-dependent resistance to PVX, was highly strain specific. Nevertheless, gene silencing mechanisms were ruled out because results from a transient expression assay implicated the 54-kD protein in the resistance mechanism (Carr and Zaitlin, 1991). In this assay, constructs designed to express the replicase caused suppression of TMV accumulation. By contrast, when the construct was expressed without an initiation codon, there was no suppression of TMV (Carr and Zaitlin, 1991). However, subsequent work revealed that homology-dependent resistance was conferred by sequences encoding the corresponding 54-kD ORF of the pepper mild mottle tobamovirus (Tenllado et al., 1995, 1996). In light of these contradictory findings from a virus related to TMV and in consideration of the fact that the 54-kD protein could not be detected in the TMV-resistant lines (Golemboski et al., 1990), the nature of the mechanism leading to resistance remains an open question in this case. It may be that the transient expression experiments (Carr and Zaitlin, 1991) did not accurately mimic the outcome in transgenic plants and that resistance in this case requires both protein-mediated and RNA homology–dependent mechanisms.

Reports of replicase-mediated resistance to PVY (Audy et al., 1994) and AlMV (Brederode et al., 1995) concluded that the resistance mechanism was protein based rather than RNA based because the resistance phenotype was influenced by mutations affecting the primary structure of the protein encoded by the transgene. Transgenes encoding wild-type versions of the replicase protein did not confer resistance, whereas those encoding mutant proteins conferred strong resistance. However, if the mutations were to cause the replicase to have a toxic effect on plant cells, there would be a bias against the recovery of primary transformants in which the transgene was expressed at a high level. Low-level transgene expressors would include those in which gene silencing and consequently the homology-dependent resistance mechanism would be active.

A particularly complex resistance phenotype was conferred by transgenic expression of a mutant 2a replicase ORF of CMV. This resistance mechanism could be dissected into independent components affecting CMV accumulation and long-distance movement (Anderson et al., 1992; Carr et al., 1994; Hellwald and Palukaitis, 1995). In some respects, the properties of this resistance resembled the RNA homology–

dependent mechanism: it was highly strain specific and was dependent on RNA sequence homology between the inoculated virus and the transgene (Zaitlin et al., 1994; Hellwald and Palukaitis, 1995). However, the suppression of virus movement is not characteristic of homology-dependent resistance and is probably a protein-mediated effect. It is likely, therefore, that the CMV transgene confers resistance through several mechanisms that may include homology-dependent resistance.

Resistance Derived from Replicating Viral Genomes

There are three reports of virus resistance in transgenic plants that express replicating viral RNAs. In the first report, tobacco plants expressing a mild strain of TMV accumulated virus particles but were symptom free and resistant to a related severe strain of TMV (Yamaya et al., 1988a, 1988b). This result suggested that transgenic expression of the viral genome mimicked the protection observed naturally when a plant is virus infected. However, it was not possible to determine whether the resistance factor in the transgenic plants was protein or RNA.

In the second report, protoplasts of plants expressing RNA1 and RNA2 of BMV exhibited BMV-specific resistance that depended on replication of the BMV RNAs (Kaido et al., 1995). There was a lower level of transgenic RNAs in the resistant lines than in the susceptible lines that expressed a similar but nonreplicable construct. This BMV resistance was likely due to homology-dependent resistance or a related process.

The third report demonstrated resistance to CMV in tobacco transformed to produce RNA1 and RNA2 of CMV. This resistance was similar to that conferred by the replicable BMV RNAs (Kaido et al., 1995) in that it required replication of the viral RNAs. However, the resistant condition was not associated with low-level accumulation of the transgene RNA and was not specific to CMV. Moreover, resistance was more effective against virion rather than RNA inocula (Suzuki et al., 1996). Therefore, it is likely that this resistance mechanism is not related to homology-dependent resistance.

FUTURE DEVELOPMENTS

Among the several categories of pathogen-derived resistance that have been described, it is not clear which may have greater applied potential than the others. The most pressing practical need is for high-level, broad spectrum resistance. This is particularly true for resistance to geminiviruses, for which there are few reports of pathogen-derived resistance (Stanley et al., 1990; Day et al., 1991; Hong and Stanley, 1996; Hong et al., 1996). Nevertheless, there is potential for the continued analysis and application of pathogen-derived resistance for disease control in plants. Future studies of CPMP may include the analysis of mutant CP transgenes to identify those that confer stronger resistance than does the wild-type CP. It may be possible to improve MPMP in a similar way, although pleiotropic effects of the transgenic MP on plasmodesmatal conductivity may complicate the resistance phenotype (Rohfritsch et al., 1996). The application of replicase-mediated resistance may also have further potential. In the one example of broad spectrum resistance obtained through transgenic expression of replicase proteins, the construct was produced unintentionally (Donson et al., 1993). Perhaps a systematic analysis of different forms of the transgenically expressed TMV RdRp will help to identify additional constructs that confer broad spectrum and high-level resistance.

A recent report of geminiviral resistance in transgenic plants illustrates a principle that may have general relevance for pathogen-derived resistance. The promoter of African cassava mosaic geminivirus was coupled to the coding sequence for dianthin, a cytotoxic protein, and the fusion construct was transformed into *N. benthamiana* (Hong et al., 1996). The transgenic viral promoter controlling dianthin production was activated in *trans* upon infection by the virus, the infected cells died, and the virus was consequently restricted to the inoculated cells. The principle underlying this work is that pathogen-derived resistance can be enhanced by fusion of the pathogen-derived sequence with sequences specifying cytotoxic or antiviral agents. The fusions could include viral promoters or *cis*-acting replication elements, as in the geminiviral work. Alternatively, the fusions could be engineered to encode proteins that would be benign in uninfected cells but that would have cytotoxic or antiviral properties after virus inoculation.

Future studies of homology-dependent resistance will continue to have a bearing on gene silencing phenomena in plants as well as on virus resistance (Baulcombe and English, 1996). An important outstanding issue concerns the identity of the factors that trigger activation of the resistance mechanism. Investigation of these factors will include rigorous tests of the threshold and aberrant RNA models and will address whether transgene methylation is a cause or a consequence of the gene silencing process. Related to this issue is the effect of RNA translatability on the activation of homology-dependent resistance. It has been suggested that untranslatable RNAs activate resistance more efficiently than do translatable RNAs (Lindbo and Dougherty, 1992b; Smith et al., 1994), but contradictory results have been obtained recently (Cassidy and Nelson, 1995; Mueller et al., 1995). The identity of sequence motifs or structures that are necessary for RNA-mediated, homology-dependent resistance is also unknown. Some viral genes are not potential targets of homology-dependent resistance (Maiti et al., 1993; Prins et al., 1996) and presumably lack these motifs or structures. In one study, the target of the homology-dependent mechanism was in the RNA sequence corresponding to the 3′ region of the transgene (English et al., 1996), indicating that the 5′ end of the RNA lacked the required structures or motifs. It will be interesting to determine whether 3′ end localization is required for other targets of homology-dependent resistance.

One topic of particular interest will be the relationship of homology-dependent resistance to the virus resistance mech-

anisms that develop naturally in virus-infected plants, producing, for example, the green island symptom. In TMV-infected plants, the green islands have only low levels of virus and are resistant to further viral infection in a strain-specific manner (Sherwood, 1988). Perhaps the green islands represent a natural manifestation of the homology-dependent resistance mechanism.

Although pathogen-derived resistance has been explored extensively, there is one strategy that has not been rigorously tested. This approach involves the transgenic expression of viral elicitors of natural host resistance responses. Natural resistance responses are a particularly useful practical resource because they often have a very broad range (Köhm et al., 1993). There are now two viral systems in which these elicitors have been identified: the TMV elicitor of *N'*-mediated resistance in tobacco (Culver and Dawson, 1991) and the PVX elicitor of *Rx*-mediated resistance in potato (Bendahmane et al., 1995). In both instances, the elicitor molecule is the viral CP. It would be interesting to determine whether controlled expression of these elicitor molecules could be engineered in transgenic plants. If the plants carry the appropriate resistance gene, it may be possible to elicit natural resistance without major perturbations to plant growth (Köhm et al., 1993). The consequence would be broad spectrum resistance to viral and perhaps to other pathogens.

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REFERENCES

Anderson, J.M., Palukaitis, P., and Zaitlin, M. (1992). A defective replicase gene induces resistance to cucumber mosaic virus in transgenic tobacco plants. Proc. Natl. Acad. Sci. USA **89,** 8759–8763.

Audy, P., Palukaitis, P., Slack, S.A., and Zaitlin, M. (1994). Replicase-mediated resistance to potato virus Y in transgenic plants. Mol. Plant-Microbe Interact. **7,** 15–22.

Barry, C., Faugeron, G., and Rossignol, J.-L. (1993). Methylation induced premeiotically in *Ascobulus*: Coextension with DNA repeat lengths and effect on transcript elongation. Proc. Natl. Acad. Sci. USA **90,** 4557–4561.

Baulcombe, D.C. (1994a). Replicase-mediated resistance: A novel type of virus resistance in transgenic plants? Trends Microbiol. **2,** 60–63.

Baulcombe, D.C. (1994b). Novel strategies for engineering virus resistance in plants. Curr. Opin. Biotechnol. **5,** 117–124.

Baulcombe, D.C., and English, J.J. (1996). Ectopic pairing of homologous DNA and post-transcriptional gene silencing in transgenic plants. Curr. Opin. Biotechnol. **7,** 173–180.

Beachy, R.N. (1993). Transgenic resistance to plant viruses. Semin. Virol. **4,** 327–416.

Beck, D.L., Guilford, P.J., Voot, D.M., Andersen, M.T., and Forster, R.L.S. (1991). Triple gene block proteins of white clover mosaic potexvirus are required for transport. Virology **183,** 695–702.

Beck, D.L., Van Dolleweerd, C.J., Lough, T.J., Balmori, E., Voot, D.M., Andersen, M.T., O'Brien, I.E.W., and Forster, R.L.S. (1994). Disruption of virus movement confers broad-spectrum resistance against systemic infection by plant viruses with a triple gene block. Proc. Natl. Acad. Sci. USA **91,** 10310–10314.

Bendahmane, A., Köhm, B.A., Dedi, C., and Baulcombe, D.C. (1995). The coat protein of potato virus X is a strain-specific elicitor of *Rx1*-mediated virus resistance in potato. Plant J. **8,** 933–941.

Brederode, F.T., Taschner, P.E.M., Posthumus, E., and Bol, J.F. (1995). Replicase-mediated resistance to alfalfa mosaic virus. Virology **207,** 467–474.

Carr, J.P., and Zaitlin, M. (1991). Resistance in transgenic tobacco plants expressing a nonstructural gene sequence of tobacco mosaic virus is a consequence of markedly reduced virus replication. Mol. Plant-Microbe Interact. **4,** 579–585.

Carr, J.P., Gal-On, A., Palukaitis, P., and Zaitlin, M. (1994). Replicase-mediated resistance to cucumber mosaic virus in transgenic plants involves suppression of both virus replication in the inoculated leaves and long-distance movement. Virology **199,** 439–447.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., and Schaad, M.C. (1996). Cell-to-cell and long-distance transport of viruses in plants. Plant Cell **8,** 1669–1681.

Cassidy, B.G., and Nelson, R.S. (1995). Differences in protection phenotypes in tobacco plants expressing coat protein genes from peanut stripe potyvirus with or without an engineered ATG. Mol. Plant-Microbe Interact. **8,** 357–365.

Chapman, S., Hills, G.J., Watts, J., and Baulcombe, D.C. (1992). Mutational analysis of the coat protein gene in potato virus X: Effects of virion morphology and viral pathogenicity. Virology **191,** 223–230.

Clark, W.G., Fitchen, J.H., Nejidat, A., Deom, C.M., and Beachy, R.N. (1995a). Studies of coat protein–mediated resistance to tobacco mosaic virus (TMV). II. Challenge by a mutant with altered virion surface does not overcome resistance conferred by TMV coat protein. J. Gen. Virol. **76,** 2613–2617.

Clark, W.G., Fitchen, J.H., and Beachy, R.N. (1995b). Studies of coat protein–mediated resistance to TMV. Virology **208,** 485–491.

Cooper, B., Lapidot, M., Heick, J.A., Dodds, J.A., and Beachy, R.N. (1995). A defective movement protein of TMV in transgenic plants confers resistance to multiple viruses whereas the functional analog increases susceptibility. Virology **206,** 307–313.

Culver, J.N., and Dawson, W.O. (1991). Tobacco mosaic virus elicitor coat protein genes produce a hypersensitive phenotype in transgenic *Nicotiana sylvestris* plants. Mol. Plant-Microbe Interact. **4,** 458–463.

Cuozzo, M., O'Connell, K.M., Kaniewski, W.K., Fang, R.-X., Chua, N.-H., and Tumer, N.E. (1988). Viral protection in transgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. Bio/Technology **6,** 549–557.

Day, A.G., Bejarano, E.R., Buck, K.W., Burrell, M., and Lichtenstein, C.P. (1991). Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus. Proc. Natl. Acad. Sci. USA **88.** 6721–6725.

de Carvalho Niebel, F., Frendo, P., Van Montagu, M., and Cornelissen, M. (1995). Post-transcriptional cosuppression of β-1,3-glucanase genes does not affect accumulation of transgene nuclear mRNA. Plant Cell **7,** 347–358.

de Feyter, R., Young, M., Schroeder, K., Dennis, E.S., and Gerlach, W. (1996). A ribozyme gene and an antisense gene are equally effective in conferring resistance to tobacco mosaic virus on transgenic tobacco. Mol. Gen. Genet. **250,** 329–338.

De Haan, P., Gielen, J.J.L., Prins, M., Wijkamp, I.G., van Schepen, A., Peters, D., van Grinsven, M.Q.J.M., and Goldbach, R.W. (1992). Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants. Bio/Technology **10,** 1133–1137.

Donson, J., Kearney, C.M., Turpen, T.H., Khan, I.A., Kurath, G., Turpen, A.M., Jones, G.E., Dawson, W.O., and Lewandowski, D.J. (1993). Broad resistance to tobamoviruses is mediated by a modified tobacco mosaic virus replicase transgene. Mol. Plant-Microbe Interact. **6,** 635–642.

Dorer, D.R., and Henikoff, S. (1994). Expansions of transgene repeats cause heterochromatin formation and gene silencing in Drosophila. Cell **77,** 993–1002.

Elmayan, T., and Vaucheret, H. (1996). Expression of single copies of a strongly expressed *35S* transgene can be silenced post-transcriptionally. Plant J. **9,** 787–797.

English, J.J., Mueller, E., and Baulcombe, D.C. (1996). Suppression of virus accumulation in transgenic plants exhibiting silencing of nuclear genes. Plant Cell **8,** 179–188.

Finnegan, E.J., Peacock, W.J., and Dennis, E.S. (1996). Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. Proc. Natl. Acad. Sci. USA **93,** 8449–8454.

Gerlach, W.L., Llewellyn, D., and Haseloff, J. (1987). Construction of a disease resistance gene using the satellite RNA of tobacco ringspot virus. Nature **328,** 802–806.

Golemboski, D.B., Lomonossoff, G.P., and Zaitlin, M. (1990). Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus. Proc. Natl. Acad. Sci. USA **87,** 6311–6315.

Goodwin, J., Chapman, K., Swaney, S., Parks, T.D., Wernsman, E.A., and Dougherty, W.G. (1996). Genetic and biochemical dissection of transgenic RNA-mediated virus resistance. Plant Cell **8,** 95–105.

Green, P.J. (1993). Control of mRNA stability in higher plants. Plant Physiol. **102,** 1065–1070.

Harrison, B.D., Mayo, M.A., and Baulcombe, D.C. (1987). Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. Nature **328,** 799–802.

Hellwald, K.-H., and Palukaitis, P. (1995). Viral RNA as a potential target for two independent mechanisms of replicase-mediated resistance against cucumber mosaic virus. Cell **83,** 937–946.

Hemenway, C.L., Fang, R.X., Kaniewski, W.K., Chua, N.-H., and Tumer, N.E. (1988). Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. EMBO J. **7,** 1273–1280.

Hobbs, S.L.A., Kpodar, P., and DeLong, C.M.O. (1990). The effect of T-DNA copy number, position and methylation on reporter gene expression in tobacco transformants. Plant Mol. Biol. **15,** 851–864.

Hobbs, S.L.A., Warkentin, T.D., and DeLong, C.M.O. (1993). Transgene copy number can be positively or negatively associated with transgene expression. Plant Mol. Biol. **21,** 17–26.

Hong, Y., and Stanley, J. (1996). Virus resistance in *Nicotiana benthamiana* conferred by African cassava mosaic virus replication-associated protein (AC1) transgene. Mol. Plant-Microbe Interact. **9,** 219–225.

Hong, Y., Saunders, K., Hartley, M.R., and Stanley, J. (1996). Resistance to geminivirus infection by virus-induced expression of dianthin in transgenic plants. Virology **220,** 119–127.

Huntley, C.C., and Hall, T.C. (1993). Minus sense transcripts of brome mosaic virus RNA-3 intercistronic region interfere with viral replication. Virology **192,** 290–297.

Ingelbrecht, I., Van Houdt, H., Van Montagu, M., and Depicker, A. (1994). Posttranscriptional silencing of reporter transgenes in tobacco correlates with DNA methylation. Proc. Natl. Acad. Sci. USA **91,** 10502–10506.

Kaido, M., Mori, M., Mise, K., Okuno, T., and Furusawa, I. (1995). Inhibition of brome mosaic virus (BMV) amplification in protoplasts from transgenic tobacco plants expressing replicable BMV RNAs. J. Gen. Virol. **76,** 2827–2833.

Kakutani, T., Jeddeloh, J.A., and Richards, E.J. (1995). Characterization of an *Arabidopsis thaliana* DNA hypomethylation mutant. Nucleic Acids Res. **23,** 130–137.

Kawchuk, L.M., Martin, R.R., and McPherson, J. (1991). Sense and antisense RNA-mediated resistance to potato leafroll virus in russet burbank potato plants. Mol. Plant-Microbe Interact. **4,** 247–253.

Köhm, B.A., Goulden, M.G., Gilbert, J.E., Kavanagh, T.A., and Baulcombe, D.C. (1993). A potato virus X resistance gene mediates an induced, nonspecific resistance in protoplasts. Plant Cell **5,** 913–920.

Kollàr, À., Dalmay, T., and Burgyàn, J. (1993). Defective interfering RNA-mediated resistance against cymbidium ringspot tombusvirus in transgenic plants. Virology **193,** 313–318.

Kumagai, M.H., Donson, J., Della-Cioppa, G., Harvey, D., Hanley, K., and Grill, L.K. (1995). Cytoplasmic inhibition of carotenoid biosynthesis with virus-derived RNA. Proc. Natl. Acad. Sci. USA **92,** 1679–1683.

Lapidot, M., Gafny, R., Ding, B., Wolf, S., Lucas, W.J., and Beachy, R.N. (1993). A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants. Plant J. **4,** 959–970.

Lawson, C., Kaniewski, W.K., Haley, L., Rozman, R., Newell, C., Sanders, P., and Tumer, N.E. (1990). Engineering resistance to mixed virus infection in a commercial potato cultivar: Resistance to potato virus X and potato virus Y in transgenic Russet Burbank. Bio/Technology **8,** 127–134.

Lindbo, J.A., and Dougherty, W.G. (1992a). Pathogen-derived resistance to a potyvirus: Immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence. Mol. Plant-Microbe Interact. **5,** 144–153.

Lindbo, J.A., and Dougherty, W.G. (1992b). Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology **189,** 725–733.

Lindbo, J.A., Silva-Rosales, L., Proebsting, W.M., and Dougherty, W.G. (1993). Induction of a highly specific antiviral state in transgenic plants: Implications for regulation of gene expression and virus resistance. Plant Cell **5,** 1749–1759.

Lomonossoff, G.P. (1995). Pathogen-derived resistance to plant viruses. Annu. Rev. Phytopathol. **33,** 323–343.

Macfarlane, S.A., and Davies, J.W. (1992). Plants transformed with a region of the 201-kilodalton replicase gene from pea early browning virus RNA1 are resistant to virus infection. Proc. Natl. Acad. Sci. USA **89,** 5829–5833.

Maiti, I.B., Murphy, J.F., Shaw, J.G., and Hunt, A.G. (1993). Plants that express a potyvirus proteinase gene are resistant to virus infection. Proc. Natl. Acad. Sci. USA **90,** 6110–6114.

Malyshenko, S.I., Kondakova, O.A., Nazarova, J.V., Kaplan, I.B., Taliansky, M.E., and Atabekov, J.G. (1993). Reduction of tobacco mosaic virus accumulation in transgenic plants producing nonfunctional viral transport proteins. J. Gen. Virol. **74,** 1149–1156.

Mueller, E., Gilbert, J.E., Davenport, G., Brigneti, G., and Baulcombe, D.C. (1995). Homology-dependent resistance: Transgenic virus resistance in plants related to homology-dependent gene silencing. Plant J. **7,** 1001–1013.

Nelson, R.S., Roth, D.A., and Johnson, J.D. (1993). Tobacco mosaic virus infection of transgenic *Nicotiana tabacum* plants is inhibited by antisense constructs directed at the 5′ region of viral RNA. Gene **127,** 227–232.

Nicholson, A.W. (1996). Structure, reactivity, and biology of double-stranded RNA. Prog. Nucleic Acid Res. Mol. Biol. **52,** 1–65.

Osbourn, J.K., Watts, J.W., Beachy, R.N., and Wilson, T.M.A. (1989). Evidence that nucleocapside disassembly and a later step in virus replication are inhibited in transgenic tobacco protoplasts expressing TMV coat protein. Virology **172,** 370–373.

Pang, S.Z., Slightom, J.L., and Gonsalves, D. (1993). Different mechanisms protect transgenic tobacco against tomato spotted wilt and impatiens necrotic spot tospoviruses. Bio/Technology **11,** 819–824.

Pang, S.Z., Jan, F.J., Carney, K., Stout, J., Tricoli, D.M., Quemada, H.D., and Gonsalves, D. (1996). Post-transcriptional transgene silencing and consequent tospovirus resistance in transgenic lettuce are affected by transgene dosage and plant development. Plant J. **9,** 899–909.

Petty, I.T.D., and Jackson, A.O. (1990). Mutational analysis of barley stripe mosaic virus RNA B. Virology **179,** 712–718.

Powell, P.A., Stark, D.M., Sanders, P.R., and Beachy, R.N. (1989). Protection against tobacco mosaic virus in transgenic plants that express tobacco mosaic virus antisense RNA. Proc. Natl. Acad. Sci. USA **86,** 6949–6952.

Powell-Abel, P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T., and Beachy, R.N. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science **232,** 738–743.

Prins, M., Resende, R.D., Anker, C., Vanschepen, A., Dehaan, P., and Goldbach, R. (1996). Engineered RNA-mediated resistance to tomato spotted wilt virus is sequence-specific. Mol. Plant-Microbe Interact. **9,** 416–418.

Ravelonandro, M., Monsion, M., Delbos, R., and Dunez, J. (1993). Variable resistance to plum pox virus and potato virus Y infection in transgenic *Nicotiana* plants expressing plum pox virus coat protein. Plant Sci. **91,** 157–169.

Register III, J.C., and Beachy, R.N. (1988). Resistance to TMV in transgenic plants results from interference with an early event in infection. Virology **166,** 524–532.

Reimann-Philipp, U., and Beachy, R.N. (1993). Coat protein–mediated resistance in transgenic tobacco expressing the tobacco mosaic-virus coat protein from tissue-specific promoters. Mol. Plant-Microbe Interact. **6,** 323–330.

Reusken, C.B.E.M., Neeleman, L., and Bol, J.F. (1994). The 3′-untranslated region of alfalfa mosaic virus RNA-3 contains at least 2 independent binding-sites for viral coat protein. Nucleic Acids Res. **22,** 1346–1353.

Rohfritsch, O., Poirson, A., Turner, A., Gagey, M.J., Roberts, K., Stussi-Garaud, C., and Godefroy-Colburn, T. (1996). A modified form of the alfalfa mosaic virus movement protein induces stressed phenotypes in transgenic tobacco. Can. J. Bot. **74,** 939–951.

Rubino, L., and Russo, M. (1995). Characterization of resistance to cymbidium ringspot virus in transgenic plants expressing a full-length viral replicase gene. Virology **212,** 240–243.

Rubino, L., Lupo, R., and Russo, M. (1993). Resistance to cymbidium ringspot virus infection in transgenic *Nicotiana benthamiana* plants expressing full-length viral replicase gene. Mol. Plant-Microbe Interact. **6,** 729–734.

Saito, T., Yamanaka, K., and Okada, Y. (1990). Long-distance movement and viral assembly of tobacco mosaic virus mutants. Virology **176,** 329–336.

Sanford, J.C., and Johnston, S.A. (1985). The concept of parasite-derived resistance—Deriving resistance genes from the parasite's own genome. J. Theor. Biol. **113,** 395–405.

Schiebel, W., Haas, B., Marinkovic, S., Klanner, A., and Sanger, H.L. (1993a). RNA-directed RNA polymerase from tomato leaves. II. Catalytic *in vitro* properties. J. Biol. Chem. **268,** 11858–11867.

Schiebel, W., Haas, B., Marinkovic, S., Klanner, A., and Sanger, H.L. (1993b). RNA-directed RNA polymerase from tomato leaves. I. Purification and physical properties. J. Biol. Chem. **268,** 11851–11857.

Sherwood, J.L. (1988). Mechanisms of cross protection between virus strains. In Plant Resistance to Viruses, D. Evered and S. Harnett, eds (Chichester, UK: John Wiley and Sons), pp. 144–157.

Sijen, T., Wellink, J., Hendriks, J., Verver, J., and Van Kammen, A. (1995). Replication of cowpea mosaic virus RNA1 or RNA2 is specifically blocked in transgenic *Nicotiana benthamiana* plants expressing the full-length replicase or movement protein genes. Mol. Plant-Microbe Interact. **8,** 340–347.

Sit, T.L., Leclerc, D., and Abouhaidar, M.G. (1994). The minimal 5′ sequence for in vitro initiation of papaya mosaic potexvirus assembly. Virology **199,** 238–242.

Smith, H.A., Swaney, S.L., Parks, T.D., Wernsman, E.A., and Dougherty, W.G. (1994). Transgenic plant virus resistance mediated by untranslatable sense RNAs: Expression, regulation, and fate of nonessential RNAs. Plant Cell **6,** 1441–1453.

Stanley, J., Frischmuth, T., and Ellwood, S. (1990). Defective viral DNA ameliorates symptoms of geminivirus infection in transgenic plants. Proc. Natl. Acad. Sci. USA **87,** 6291–6295.

Suzuki, M., Masuta, C., Takanami, Y., and Kuwata, S. (1996). Resistance against cucumber mosaic virus in plants expressing the viral replicon. FEBS Lett. **379,** 26–30.

Swaney, S., Powers, H., Goodwin, J., Rosales, L.S., and Dougherty, W.G. (1995). RNA-mediated resistance with nonstructural genes from the tobacco etch virus genome. Mol. Plant-Microbe Interact. **8,** 1004–1011.

Taschner, P.E.M., Van Marle, G., Brederode, F.T., Turner, N.E., and Bol, J.F. (1994). Plants transformed with a mutant alfalfa mosaic virus coat protein gene are resistant to the mutant but not to wild-type virus. Virology **203,** 269–276.

Tenllado, F., Garcia-Luque, I., Serra, M.T., and Diaz-Ruiz, J.R. (1995). *Nicotiana benthamiana* plants transformed with the 54-kDa region of the pepper mild mottle tobamovirus replicase gene exhibit two types of resistance responses against viral infection. Virology **211,** 170–183.

Tenllado, F., Garcia-Luque, I., Serra, M.T., and Diaz-Ruiz, J.R. (1996). Resistance to pepper mild mottle tobamovirus conferred by the 54-kDa gene sequence in transgenic plants does not require expression of the wild-type 54-kDa protein. Virology **219,** 330–335.

Van Blokland, R., Van der Geest, N., Mol, J.N.M., and Kooter, J.M. (1994). Transgene-mediated suppression of chalcone synthase expression in *Petunia hybrida* results from an increase in RNA turnover. Plant J. **6,** 861–877.

Van der Vlugt, R.A.A., Ruiter, R.K., and Goldbach, R.W. (1992). Evidence for sense RNA-mediated protection to PVY^N in tobacco plants transformed with the viral coat protein cistron. Plant Mol. Biol. **20,** 631–639.

Wassenegger, M., Heimes, S., Riedel, L., and Sänger, H.L. (1994). Methylation of plant genome–integrated viroid cDNAs is induced upon replication of the corresponding viroid RNA: A novel mechanism of an RNA-directed sequence-specific *de novo* methylation of genes. Cell **76,** 567–576.

Wilson, T.M.A. (1993). Strategies to protect crop plants against viruses: Pathogen-derived resistance blossoms. Proc. Natl. Acad. Sci. USA **90,** 3134–3141.

Wisniewski, L.A., Powell, P.A., Nelson, R.S., and Beachy, R.N. (1990). Local and systemic spread of tobacco mosaic virus in transgenic tobacco. Plant Cell **2,** 559–567.

Yamaya, J., Yoshioka, M., Meshi, T., Okada, Y., and Ohno, T. (1988a). Expression of tobacco mosaic virus RNA in transgenic plants. Mol. Gen. Genet. **211,** 520–525.

Yamaya, J., Yoshioka, M., Meshi, T., Okada, Y., and Ohno, T. (1988b). Cross protection in transgenic tobacco plants expressing a mild strain of tobacco mosaic virus. Mol. Gen. Genet. **215,** 173–175.

Yepes, L.M., Fuchs, M., Slightom, J.L., and Gonsalves, D. (1996). Sense and antisense coat protein gene constructs confer high-levels of resistance to tomato ringspot nepovirus in transgenic *Nicotiana* species. Phytopathology **86,** 417–424.

Zaccomer, B., Cellier, F., Boyer, J.C., Haenni, A.L., and Tepfer, M. (1993). Transgenic plants that express genes including the 3′ untranslated region of the turnip yellow mosaic virus (TYMV) genome are partially protected against TYMV infection. Gene **136,** 87–94.

Zaitlin, M., Anderson, J.M., Perry, K.L., Zhang, L., and Palukaitis, P. (1994). Specificity of replicase-mediated resistance to cucumber mosaic virus. Virology **201,** 200–205.

Ziegler-Graff, V., Guilford, P.J., and Baulcombe, D.C. (1991). Tobacco rattle virus RNA-1 29K gene product potentiates viral movement and also affects symptom induction in tobacco. Virology **182,** 145–155.